UNITED ST. DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/321,655	05/28/99	GERSON S	640100-304

HM12/0316

ELLIOT M OLSTEIN ESQ
C O CARELLA BYRNE BAIN GILFILLAN
CECCHI STEWART & OLSTEIN
6 BECKER ROAD
ROSELAND NJ 07068

EXAMINER	
NGUYEN, Q	
ART UNIT	**PAPER NUMBER**
1632	

DATE MAILED: 03/16/00

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary	Application No.	Applicant(s)	
	09/321,655	GERSON, STANTON L.	
	Examiner	Art Unit	
	Quang Nguyen, Ph.D.	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).

Status

1)☐ Responsive to communication(s) filed on _____ .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) 1-4 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) 1-4 is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claims _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

a)☐ All b)☐ Some * c)☐ None of the CERTIFIED copies of the priority documents have been:

1.☐ received.

2.☐ received in Application No. (Series Code / Serial Number) _____ .

3.☐ received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. & 119(e).

Attachment(s)

14) ☒ Notice of References Cited (PTO-892)	17) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
15) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)	18) ☐ Notice of Informal Patent Application (PTO-152)
16) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .	19) ☐ Other: .

DETAILED ACTION

Priority

This application claims priority on U.S. Provisional Application No. 60/087284, filed 05/29/98.

Applicant has not complied with one or more conditions for receiving the benefit of an earlier filing date under 35 U.S.C 119(e). An application in which the benefits of an earlier application are desired must contain a specific reference to the prior application(s) in the first sentence of the specification (37 CFR 1.78). The first sentence of the specification does not reference the claimed provisional application. The first line of the specification should read: "This application claims the benefit of U.S. Provisional Application No. 60/087284, filed 05/29/98.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> **The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.**

Claims 1-4 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of transducing human hematopoietic progenitor cells *in vitro* with retroviral vectors coding for O^6-alkylguanine-DNA alkyltransferase (AGT) gene or its mutant forms in a co-culture with human mesenchymal stem cells which are either autologous or allogeneic to the hematopoietic

progenitor cells, and the same method further comprising the separation of the two transduced stem cell populations for *in vitro* and animal studies, does not reasonably provide enablement for the method of transducing any and all genes in hematopoietic progenitor cells in a co-culture with human mesenchymal stem cells for use in human gene therapy, and the same method further comprising the separation of transduced hematopoietic progenitor cells from human mesenchymal stem cells. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

The factors to be considered in the determination of an enabling disclosure have been summarized as the quantity of experimentation necessary, the amount of direction or guidance presented, the state of the prior art, the relative skill of those in the art, the predictability or unpredictability of the art and the breadth of the claims. *Ex parte Forman*, (230 USPQ 546 (Bd Pat. Appl & Unt, 1986); *In re Wands*, 858 F.2d 731, 8 USPQ 2d 1400 (Fed. Cir. 1988)).

Claims 1-4 are drawn to a method of transducing hematopoietic progenitor cells with retroviral vectors encoding exogenous genetic material in a co-culture with human mesenchymal stem cells wherein the mesenchymal stem cells are either autologous or allogeneic to the hematopoietic progenitor cells. The method further involves the separation of the two transduced stem cell populations. When read in light of the specification, the claims encompass a method to generate haematopoietic and

mesenchymal stem cells transduced with any and all therapeutic agents for human gene therapy.

The specification discloses a method wherein human CD34 hematopoietic progenitor cells are transduced *in vitro* with a retroviral vector coding for a mutant DNA repair enzyme AGT gene under various conditions, including: (a) a co-culture with human mesenchymal stem cells (allogeneic or autologous), (b) in flasks coated with fibronectin fragment, and (c) in flasks coated with Dexter stroma. Examples show that co-cultured human mesenchymal stem cells support the transduction efficiency, cell expansion, expression of mutant AGT, and resistance to drug treatment for transduced CD34 hematopoietic progenitor cells. Fibronectin fragment and Dexter stroma, previously known to enhance gene transduction efficiency for human hematopoietic progenitor cells, demonstrate similar results. Such evidence is noted and considered, however, can not be extrapolated to the claimed invention which is directed to a method of transducing any and all therapeutic genes in hematopoietic progenitor cells and human mesenchymal stem cells for human gene therapy. The specification is not enabled for such a broad claim because at the effective filing date of the instant application, gene therapy was an unpredictable art. In a recent meeting report on a workshop for gene therapy and translational cancer research (Clin. Cancer Res. 5:471-474, 1999), Dang et al. noted that further advancement in all fields including, gene delivery, gene expression, immune manipulation, and the development of molecular targets is needed to make gene therapy a reality. They further cited the findings of the

Orkin-Motulsky Committee (commissioned by the NIH director) which found that human gene therapy is an immature science with limited understanding of gene regulation and disease models for preclinical studies (First paragraph, page 471). Dang et al. pointed out several factors limiting an effective human gene therapy, including, suboptimal vectors, the lack of long term and stable gene expression, and most importantly the efficient gene delivery to target tissues (last paragraph, page 474). Eck and Wilson (Gene-based therapy, 1996) discussed further in details several factors that complicate *in vivo* gene transfer and expression which result in therapeutic effects. These include, the fate of delivering vectors, the fraction of vectors taken up by the target cell population, the rate of vector degradation, the level of mRNA produced, the stability of the protein produced, the protein's compartmentalization within the cell or its secretory fate (Column 1, page 82). The above factors differ dramatically based on the protein being produced, and the desirable therapeutic effect being sought. Therefore, the level of gene expression, its duration, and its *in vivo* therapeutic effects are not always predictable, and hence not shown to be overcome with routine experimentation.

The specification of the instant application fails to provide any teaching or evidence demonstrating that aforementioned obstacles for an effective human gene therapy can be overcome. It simply recites the use of all retroviral vectors, promoters, and gene transfer methods available in the art (pages 7-8). The specification also discloses in broad and general terms various exogeneous genes to be expressed in transduced hematopoietic progenitors and human mesenchymal stem cells to treat

unspecified genetic disorders and diseases of the blood and immune system. Furthermore, it recites that the transduced stem cells are to be administered by various methods that include, infusion, direct depot injection into periosteal, bone marrow and subcutaneous sites (Second paragraph, page 9).

At the time of filing for the instant application, cell transplantation therapies with genetically altered cells to treat diseases and disorders are neither routine nor predictable. Prockop (Science, 276:71-74, 1997) indicated several different strategies that are being pursued for therapeutic use of mesenchymal stem cells, including that involving mesenchymal stem cells that are genetically modified to secrete a therapeutic gene. However, he noted that "a number of fundamental questions about mesenchymal stem cells still need to be resolved before they can be used for safe and effective cell and gene therapy". Similarly, regarding to the utilization of mesenchymal stem cells for human gene therapy, Gerson (Nature Med. 5:262-264, 1999) indicated many questions that need to be addressed, such as, "What is the minimum proportion of donor mesenchymal stem cells required to affect a long-lasting therapeutic response?", "Will transplantation of mesenchymal stem cells from a marrow harvest or from culture-expansion be sufficient to treat other diseases?", "Can culture-expanded mesenchymal stem cells substitute for fresh marrow allografts in the correction of genetic disorders of the mesenchyme?", "To which host tissues do infused mesenchymal stem cells home, proliferate and differentiate, and using which regulatory signals?", "Can mesenchymal stem cells be used effectively for gene transfer and gene deliver?", "Is systemic infusion

optimal or is infusion into a target organ required?" (column 1, second paragraph, page 264).

More recently, Koc et al. (J. Clin. Oncology 18:307-316, 2000) reported phase I and II clinical results which demonstrated that autologous mesenchymal stem cells can be culture-expanded, infused along with peripheral blood progenitor cells in advanced breast cancer patients treated with high dose chemotherapy without any toxicity, and they are associated with rapid hematopoietic recovery in patients (Abstract, page 307). However, the authors noted that the distribution, survival, and participation of culture-expanded mesenchymal stem cells in tissue function in treated patients is largely unknown (column 2, first paragraph, page 315). Koc et al. further indicated that there are on-going studies investigating the safety of allogeneic culture-expanded mesenchymal stem cell infusion in humans, and those involving therapeutic-gene transduced mesenchymal stem cells are currently under development. Similarly, clinical trials involving autologous transplantation of hematopoietic stem cells transduced with a retrovirus containing the Multidrug Resistance gene (MDR-1) in metastatic breast cancer patients revealed that the overall transduction efficiency, long-term stable engraftment of gene-modified hematopoietic stem cells, and their *in vivo* selection remain challenges need to be overcome to make an effective gene therapy a reality (Deisseroth, Clin. Cancer Res. 5:1607-1609, 1999).

Accordingly, due to the absence of working examples, the unpredictability of the human gene therapy art, the many variable factors controlling an effective human gene

therapy, and the breadth of the claims, it would have required undue experimentation for one skilled in the art to make and use the broadly claimed invention.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1 and 4 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

In claim 1, it is unclear what is meant by the phrase "in the presence of", does it mean that hematopoietic progenitor cells are co-cultured with mesenchymal stem cells? Furthermore, it is not clear what is encompassed by the phrase "exogenous genetic material". Does it include genetic material in any form (naked DNA, liposomes, bacterial vectors, etc.) and from any source (allogeneic and xenogeneic)? Clarification is needed.

Claim 4 recites the limitation "human progenitor cells" in line 1 of claim 1. There is insufficient antecedent basis for this limitation in the claim. Claim 1 recites only hematopoietic progenitor cells, not human progenitor cells.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1, 3, and 4 are rejected under 35 U.S.C. 102(b) as being anticipated by Reese et al. (Proc. Natl. Acad. Sci. 93:14088-14093, 1996).

Claim 1 is drawn to a method of transducing hematopoeitic progenitor cells with a retroviral vector encoding and expressing a non-endogenous gene in the presence of human mesenchymal stem cells. The mesenchymal stem cells can be derived from mononuclear cells isolated from the marrow, and they can be either irradiated or non-irradiated prior to use (Specification, line 1, page 5, lines 9-12, page 6, line 31, page 9). Claims 3 and 4 are drawn to said method wherein the mesenchymal stem cells are allogeneic to the hematopoietic progenitor cells, and further comprising the separation of the two said cell populations, respectively.

Reese et al. disclosed a retroviral transduction of a mutant methylguanine DNA methyltransferase gene into human CD34 cells (hematopoietic progenitor cells as defined by the specification on line 29, page 4) which are resistant to a combination of O^6-benzylguanine and 1,3-bis(2-chloroethyl)-1-nitrosourea (Abstract). The transduction was carried out in CD34 cells cocultured on a stroma generated by bone marrow mononuclear cells that were subsequently irradiated prior to the plating of CD34 cells (column 2, first paragraph, page 14089). Since there was no separation between the bone marrow stroma and irradiated bone marrow mononuclear cells prior to use, and this cell population encompasses mesenchymal stem cells, CD34 cells were in effect

transduced in the presence of irradiated human mesenchymal stem cells. The reference did not disclose whether human bone marrow mononuclear cells were derived from the same adult patients from whom CD34 cells were obtained. Therefore, the used mesenchymal stem cells are allogeneic to the hematopoietic progenetor cells. Furthermore, Reese et al. disclosed that the transduced CD34 cells were removed from the stromal layer containing irradiated mesenchymal stem cells with cell dissociation buffer (last sentence, first paragraph, column 2, page 14089). Thus, the reference anticipates the claimed invention.

Claims 1, 3, and 4 are rejected under 35 U.S.C. 102(b) as being anticipated by Nolta et al. (Blood 86:101-110, 1995).

Nolta et al. disclosed a transduction method for human CD34 cells isolated from bone marrow and peripheral blood with retroviral vectors containing either the bacterial neo gene, or normal human glucocerebrosidase in the presence of a stroma generated by human allogeneic stromal cells which were irradiated prior to the plating of CD34 cells (Abstract, and column 1, page 102). The utilized mesenchymal stromal cell population derived from bone marrow spicules is devoid of most hematopoietic cells (column 1, third paragraph, page 102), and may contain mesenchymal stem cells. Therefore, Nolta et al. described a transduction method for CD34 cells in the presence of human allogeneic mesenchymal stem cells. The reference further disclosed the isolation of transduced, nonadherent CD34 cells after the transduction by vigorous

flushing and plating the collected cells twice to eliminate adherent stromal cells (column 1, last paragraph, page 102). Thus, the reference anticipates the claimed invention.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-4 are rejected under 35 U.S.C. 103(a) as being unpatentable over Reese et al. (Proc. Natl. Acad. Sci. 93:14088-14093, 1996) in view of Gerson et al. (U.S. Patent No. 5591625, issued date 1/7/1997).

The claims are drawn to a method of transducing the hematopoietic progenitor cells with retroviral vectors containing non-endogenous gene in a co-culture with isolated human mesenchymal stem cells (Claim 1). The said transducing method wherein the isolated mesenchymal stem cells are either autologous (Claim 2) or allogeneic (Claim 3) to the hematopoietic progenitor cells. Claim 4 is drawn to the transducing method in claim 1 that is further comprising the separation of the transduced progenitor cells from the human mesenchymal stem cells.

Reese et al. disclosed a transducing method for human hematopoietic CD34 progenitor cells plated on a stroma containing irradiated allogeneic bone marrow stromal cell population that may include mesenchymal stem cells, as previously

discussed. Reese et al. did not teach the transduction of an exogenous gene in hematopoietic progenitor cells in a co-culture .with isolated allogeneic or autologous human mesenchymal stem cells. Nor did the reference disclose a separation of transduced hematopoietic progenitor cells from the human mesenchymal stem cells.

However, at the time the claimed invention was made, Gerson et al. had disclosed the isolation and culture of a homogeneous human mesenchymal stem cell population derived from normal bone marrow of normal donors (allogeneic) or cancer patients undergoing autologous bone marrow harvest. (Example 2, column 3). The harvested human mesenchymal cells are fusiform adherent cells with multiple projections, with surface antigens recognized by SH2, SH3 and SH4 monoclonal antibodies, and free of hematopoietic progenitor cell contamination (column 14, third paragraph).

Accordingly, it would have been obvious to a person of ordinary skill in the art at the time of invention was made to modify the transducing method of human hematopoietic CD34 progenitor cells described by Reese et al. by substituting a stroma containing irradiated allogeneic bone marrow stroma cells with isolated homogeneous human mesenchymal stem cells as taught by Gerson et al.. The human mesenchymal stem cells can be either allogeneic or autologous to the transfected human hematopoietic progenitor cells. Furthermore, the transduced method of Reese et al. can include isolation steps described by Gerson et al. to separate transduced human hematopoietic progenitor cells from human mesenchymal stem cells. One would have

been motivated to modify the transduced method of Reese et al. with changes set forth above, to investigate the role of isolated human mesenchymal stem cells that share some similar characteristics to a bone marrow stromal cell population frequently used as feeder cells, in the transduction and long-term culturing of human hematopoietic progenitor cells. In addition, one would be able to obtain both hematopoietic progenitor cells and mesenchymal stem cells transducing exogenous gene products of physiological and pharmacological values for gene therapeutic purposes in a single transfection method with reasonable expectation of success. Thus, the claimed invention as a whole was clearly prima facie in the absence of evidence to the contrary.

Claims 1-4 are rejected under 35 U.S.C. 103(a) as being unpatentable over Nolta et al. (Blood 86:101-110, 1995) in view of Gerson et al. (U.S. Patent No. 5591625, issued date 1/7/97).

Nolta et al. disclosed a transduction method for human CD34 cells isolated from bone marrow and peripheral blood with retroviral vectors containing either the bacterial neo gene, or normal human glucocerebrosidase on a stroma containing irradiated human allogeneic stromal cells. Nolta et al. did not teach a transducing method for hematopoitic progenitor cells in a co-culture with isolated homogeneous human mesenchymal stem cells which are either autologous or allogeneic to the transduced hematopoietic progenitor cells, and steps in said method to separate the two transduced cell populations.

The teachings of Gerson et al. has been discussed previously.

Accordingly, it would have been obvious to a person of ordinary skill in the art at the time of invention was made to modify the transducing method of Nolta et al. by substituting a stroma containing irradiated allogeneic stroma cells with isolated homogeneous human mesenchymal stem cells, and by incorporation of the isolation steps for human mesenchymal stem cells as taught by Gerson et al. to arrive at the instant claimed invention. The motivation to carry out the modified transducing method is the same as that to modify the transducing method of Reese et al., as set forth above. Thus, the claimed invention as a whole was clearly prima facie in the absence of evidence to the contrary.

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Quang Nguyen, Ph.D., whose telephone number is (703) 308-8339.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jasemine C. Chambers, Ph.D., may be reached at (703) 308-2035.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-2801.

To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1632.

Papers related to this application may be submitted to Group 160 by facsimile transmission. Papers should be faxed to Group 160 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15,1989). The CM1 Fax Center number is (703) 305-3014 or (703) 308-4242.

Jasemine C. Chambers

JASEMINE CHAMBERS
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600